UNIVERSITY OF PENNSYLVANIA

CHROMOSOME STRUCTURE AND ITS RELATION TO THE CHROMOSOME CYCLE

I. SOMATIC MITOSES IN *TRADESCANTIA PILOSA*

BERWIND P. KAUFMANN

A THESIS

IN BOTANY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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CHROMOSOME STRUCTURE AND ITS RELATION TO THE CHROMOSOME CYCLE

I. SOMATIC MITOSES IN *TRADESCANTIA PILOSA*

Berwind P. Kaufmann

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While studying the structure of the chromosomes of *Tradescantia pilosa* Lehm. during the maturation phases, details of chromatin organization and arrangement were encountered which required for their elucidation a critical analysis of the somatic mitoses. One of the most perplexing problems concerned the almost constant arrangement of the chromatin in the form of spiral threads. There is nothing new in the discovery of such spiral bands in plant chromosomes, but considerable controversy has arisen as to their possible function and relation to the chromosome cycle. Having observed the chromatic spiral threads in the chromosomes of all the phases of the first maturation division, the question naturally arose as to whether such organization was restricted to meiosis. For an answer it was necessary to consider both the somatic and premeiotic nuclei, which were alike in most details. The relative ease with which root tips could be obtained made it advantageous to work with them. Figures of premeiotic cells have been included only to corroborate certain features. Discussion of the meiotic phases will be reserved for a subsequent publication.

The divergence of the cytological observations so often presented by different workers on the same cells is enough to show that any effort to discern the smaller visible elements of chromosomes must require the application of a variety of fixatives in lengthy series, so that the different appearances produced may be properly evaluated. This is particularly true of Tradescantia, which has been noted for the difficulties it presents in obtaining adequate fixation. Referring to *T. virginiana*, Strasburger (1900) remarked, "Zwar lassen sich die karyokinetischen Bilder in diesen Pollenmutterzellen im Allgemeinen nicht gut fixiren." Farmer and Shove (1905) stated that similar difficulties were encountered. Sharp (1920), studying the somatic mitoses of the same species, commented on the inferiority of his preparations of this material for late prophase stages as compared with

those of Vicia. However, he regarded Tradescantia as superior material for analysis of the critical stages of the telophases and early prophases.

The tediousness of the paraffin method has been an important factor in curtailing efforts to obtain critical fixation. For eliminating that technique I am particularly indebted to the smear method recently described by Taylor (1924). This allowed the application of a lengthy series of fixatives in much less time than would have been required by the paraffin or the celloidin method. Although designed for the study of the developing microsporocytes, the smears disclosed dividing tapetal cells of the microsporangium in sufficient quantity to give some idea of the value of the fixative for somatic mitoses. Such values were only an index, however, and the fixatives usually required certain alterations of the proportions of their constituent chemicals before being entirely adaptable to the paraffin method. This is in harmony with McClung's (1918) comment that a fixative which produces good smear preparations may give atrocious results with even a reasonable mass of tissue.

No one formula can be detailed as that of a universal fixative. During two growing seasons and a portion of a third the cells of this plant were fixed in various modifications of a number of types of killing agents, with the apparent result that an excellent preservative of the elements during one phase often proved quite destructive to those elements during another phase. The best fixations were obtained with chrom-osmic-acetic acid combinations, to which such adjuvants as lactose, maltose, and urea were added.

Staining of the preparations used for the following study was done by the iron-alum haematoxylin method. For purposes of viewing the details of those stages wherein the chromosomes have the greater affinity for the dye, such as the metaphases and anaphases, it was found necessary, as shown by Lee (1920) and Martens (1922), to curtail considerably the period of staining. I found that a period of staining of one hour or less in a one fourth to one half percent solution of haematoxylin, following mordanting for an equal time in two and one half percent iron alum, gave a density of stain which could readily be differentiated. The alternative method, of staining six to twelve hours in a one half percent solution and differentiating for a sufficient period of time to bring out the desired structure, more often resulted in a brownish black or muddy color than in the desired clarity.

I am greatly indebted to Dr. W. R. Taylor, under whose direction this problem was conducted, and to Dr. E. Eleanor Carothers, Prof. Clarence E. McClung, and Dr. William Seifriz for valuable suggestions and criticisms.

Historical

Study of chromosome structure in members of the genus Tradescantia dates back to the work of Baranetzky (1880). His investigations concerned

T. virginica, *T. pilosa*, *T. subaspera*, *T. discolor* (*Rhoeo discolor*), and *T. zebrina*. Because he did not like the fixation appearances of material preserved in alcohol, Baranetzky confined his observations to living cells Anthers were crushed under the cover glass and the extruded cells were studied in spring or in salt water. Although Baranetzky described the nuclear thread as having the appearance of alternating discs of darker and lighter material, this was the aberrant condition. A more critical interpretation found the thread composed of two substances, with the denser confined to the superficial layer and in the form of a spiral band, which view was verified not only by the observation of the coiled but also by that of the uncoiled thread (Baranetzky's figs. 41 *a*, *b*, *c*, *d*, *f*, *h*). Baranetzky realized that such definite structure was perhaps indicative of a fundamental functional relationship: "Ob der dichten Spiralfaser dabei wirklich irgend eine mechanische Rolle zukommt, mögen spätere Untersuchungen entscheiden."

Strasburger (1882) did little work on living objects, but drew his conclusions from fixed material. His observations on pollen mother cells of *T. subaspera* revealed a nuclear thread composed of alternating discs of darker material and lighter "Zwischensubstanz." To these he applied the terms "Nucleo-mikrosomensubstanz" and "Nucleo-hyaloplasma." He admitted his inability to see a tightly wound spiral and reported unsuccessful efforts to confirm Baranetzky's experiment of uncoiling such a spiral. He gave no figures of Tradescantia in this paper, but his figure 73 *b* of *Fritillaria imperialis* illustrates the type of chromosome structure described.

Miyake (1905) also limited his observations to the meiotic phases. He noted that, so long as study was restricted to fixed material, absolute certainty could not be attached to the interpretations. His figure 148 of a metaphase of the first maturation division gives some indication of internal chromosome structure, although it is illustrative of chromomeres or "Körnige Struktur" and not of a spiral thread.

Farmer and Shove (1905) described both somatic and meiotic phases in *T. virginica*. The arrangement of the chromatin within the linin filament of the early somatic prophases was of "the intermittent character to which the appearance of alternate stainable and non-stainable discs is due." The chromatin discs or granules were later presumed to split in order to produce the longitudinal fission of the chromosome.

Van Herwerden (1910), verifying Baranetzky's observations, studied *T. zebrina* by expressing the nuclear thread from the cell into dilute acetic acid and methyl green. She noted that it would be difficult to describe these threads as other than spiral, although their size was not so favorable for study as in the case of the Chironomus larva, a consideration of which formed the bulk of the paper. She thought the spiral is but a temporary phase of the mitotic division in Tradescantia, whereas in Chironomus it is a persistent structure.

A more recent contribution, restricted entirely to chromosome structure, particularly of *T. virginiana*, is that of Sands (1923). Somatic as well as meiotic chromosomes were described and figured. Sands employed primarily Belling's aceto-carmine method, although fixed material and pollen mother cells teased out on a slide into three-percent cane sugar solution were also used. In the aceto-carmine preparations, "the structure came out with the first swelling, somewhat as does the image on a photographic plate, without any perceptible change either in the form or in the position of the elements from that seen in the living condition." The chromosome was pictured as a linin cylinder within which the chromatin material is peripherally disposed. The latter exists as chromomeres, and these are variable in size, shape, and number. Indications that they may assume the appearance of a spiral thread were acknowledged by Sands when he said that "sometimes they show traces of a spiral arrangement (figs. 17, 18, Pl. XXX)."[1] The linin substratum was regarded as of jelly-like consistency, and in it the chromatin was considered to be imbedded. The colloidal structure was considered as primarily a two-phase system with the linin representing the continuous and the chromatin the disperse phase.

The most critical analysis of somatic mitoses was made by Sharp (1920) on root tips of *T. virginiana*. He described a telophasic vacuolation transforming the chromosomes into alveolar-reticulate structures. In the following prophases a single chromatic thread appears to be formed from each of the chromosomes. The splitting of that thread was described as occurring by means of the formation of a series of vacuoles, most of which were regarded as new formations and not as structures persisting from the preceding telophases. The true splitting of the chromosomes was therefore considered to be a prophase development and not to be traced back to the telophasic vacuolation.

Observations

Anaphases

At the inception of the anaphases the chromosomes arch towards the poles, forming long V's, the median constriction of each serving as the point of spindle-fiber attachment. The median constriction is a zone of variable width, sometimes recognizable only as a narrow slit (Pl. VI, fig. 1), at other times appearing as a conspicuous area between the chromosome arms (fig. 2). Under proper differentiation of stain it appears as a grayish-black zone of less diameter than the denser black arms. Robertson (1916) has applied the term "achromatic bridges" to such points of spindle-fiber attachment found in V-shaped chromosomes. This designation is applicable to the anaphase chromosomes of *T. pilosa*, since they present evidence of differentiation into two dissimilar substances to which the terms *chromatic*

[1] These are photomicrographs of pollen mother cells of *Rhoeo discolor*, in the metaphases of the first meiotic division. In these cells I have seen the same type of spiral structure to be described for *Tradescantia pilosa*.

and *achromatic* may be applied. Martens (1922) has observed a similar differentiation within the chromosomes of *Paris quadrifolia*, but he notes and figures the achromatic material as showing some affinity for the stain. This is equally true of my preparations. The terms *achromatic* and *chromatic* therefore become merely relative and replaceable by the terms *oxychromatin* and *basichromatin*, with which they are here regarded as synonymous. Where differentiation of the stain is not sufficient, the achromatic and chromatic materials present such a homogeneity of color that the constriction, if recognizable at all, is distinguished only by its comparative slenderness. Apparently the two systems of the chromosome have at anaphase a more nearly equal affinity for the stains than during some of the other phases. This fact necessitated the method of staining described above. Using such a method, the chromosome appears no longer a homogeneous unit. Its end view (fig. 3) suggests a hollow ring with a narrow lumen, showing that the more densely staining material is peripherally disposed. The lumen is actually a space between the ends of two chromatic threads which can be seen as intertwined spirals when the chromosome is viewed laterally (fig. 4). Since these spirals are homologous with the *chromonema* of Vejdovský (1912), as will be demonstrated later, it would perhaps be well to recognize the term *chromonematic substance* used by Martens as synonymous with the term *chromatic substance*. Between the spiral threads can be recognized the lighter-colored achromatic material. Such observations suggest that the chromosome is essentially a long rod of achromatic material, of which the median constriction is a part and within which is peripherally disposed the chromonematic material. Further verification of this composite structure is offered by such a chromosome as that shown in figure 5. This was taken from a cell several layers below the surface of the root, where penetration of the fixative was hindered sufficiently to allow of distortion and breaking of the chromonemata. Accordingly, the aspect figured must not be regarded as a criterion of delicate structure perfectly preserved, but it is valuable in demonstrating the existence of the two systems composing the chromosome. There is also the suggestion of a limiting membrane to this chromosome, a condition exaggerated quite beyond the normal, in which condition membranes can not be seen to occur. But failure to see them does not offset the probability of their existence, as was suggested by Seifriz (1921).

That the chromatic substance really exists at this stage in the form of spiral threads is verified by the following evidence: (*a*) Even when the stain is too deep to permit a visible differentiation of the substances of the chromosomes, the undulating contour and the resultant moniliform appearance suggest a definite internal organization. There can be no question that such an outline would be formed by two intertwined spiral threads. Where the threads appear to cross, that is, where they are lying one above the other, the chromosome appears narrower. In the gradual transition

from that region to the one where the threads appear to lie side by side, the chromosome outline gradually broadens, to constrict once more as the threads appear to recede from the common plane. Such a moniliform aspect can be observed in any lateral view (figs. 6, 12). Bendings, torsions, and foreshortening naturally do much to offset the maximum diagrammatic appearance. (*b*) The chromosome often shows a terminal indentation or notch which is highly suggestive of the beginning of a longitudinal split. Projecting slightly on each side of the notch is the end of a thread (figs. 4, 6, 7). (*c*) Torn ends of chromosomes occasionally show two projecting threads. Sometimes their angle of divergence is great enough to offset any doubt as to the duality of structure involved (fig. 8). (*d*) When the chromosome can be visibly analyzed, the chromonemata can be traced. In staining for these structures, the achromatin colors slightly or not at all, so that where the chromatin can be most clearly observed the achromatic material is often indistinguishable. Figures 4, 9, 10, and 11 demonstrate the continuity of the chromonemata. There is apparently no preciseness to the pitch of the spiral in different chromosomes or even in the same one. Loose coils seem more abundant than tight ones, and this is probably because of the extreme length of the chromosomes, the origin of which can be traced back to their behavior during the prophase. It can be determined that in some cases the helix is dextrorsal and in other cases sinistrorsal. I could not determine whether the number of chromosomes representing each of these types was equal or not. It is clear that, since the cleft between the chromonemata here follows a spiral and not the long axis of the chromosome, it can safely be accepted as an actual rift and not an apparent one.

Satellites occasionally are seen in these anaphase cells. The satellite is a most delicate terminal appendage, seemingly imbedded in the apex of a conical projection of achromatic material (figs. 14, 15). From the limited number of specimens clearly illustrating these structures it appears as if but a single satellite exists on one arm of one of the chromosomes moving toward a pole (fig. 6).

As migration to the poles proceeds (fig. 12), it is usually impossible to detect spindle fibers, owing perhaps to the type of fixative and stain employed. A gradual shortening and thickening of the chromosome arms occurs. This is possibly accomplished by the tightening of the spirals, for many of these chromosomes give indications of more closely wound threads than during the earlier stages (fig. 10). Many of the typical V's are altered by the bending of one or both of the arms. Occasionally it seems as if a certain amount of untwisting of the chromosome occurs, which results in an extension of at least part of the spiral (fig. 16). This chromosome is particularly interesting, not only because of the verification of the longitudinal split between the halves, but also because of the suggestion of chromomeres. These can be noted as more darkly staining zones of

greater diameter than the rest of the thread, so that they stand out as bulges or swellings. The fact that they as well as the chromonemata are paired, seems to indicate that on further investigation the type of organization here involved might prove to be as precise as that detailed by Wenrich (1916) for certain chromosomes of Phrynotettix.

Continued shortening occurs until the chromosomes approximate at the poles. From the upper group of figure 18 it would appear as though clumping had occurred to the extent of obliterating the boundaries of the component individuals. It seems to me, however, that this grouping is either an artifact or an optical effect due to the mass of the material and the density of the stain. This premise is strengthened by the appearance of the lower group of the same cell and by figure 17. In these cases the members of the complex can be recognized as maintaining their individuality. More specific evidence is offered by the structure within the chromosome. Even when clumping does seem to occur, a projecting member or a portion of one may reveal the chromonemata. Thus, chromosome *a* of figure 18, detailed in figure 19, shows a tightly wound coil, suggesting that the shortening may in part be accomplished by the tightening. Projecting ends of two chromosome arms are also shown in figure 20. If an isolated individual is considered, as in figure 21, the notched ends and twisted threads indicate that the continuity of the chromonemata has not been effaced. Neither is there any suggestion that the chromatic and achromatic systems have lost their identities.

The fibers between the two groups of chromosomes are now pronounced, and the cell plate is beginning to differentiate across the fibers at approximately a median position.

Telophases

There is presented, to complicate the analysis of the telophase activity, a series of changes concerned with the transition of the chromophilous anaphase chromosomes into the delicate threads of the late telophases and the apparent reticulum of the interphases. Very early in this process a limiting membrane is established around the nucleus, separating its contents from the cytoplasm. This membrane can be seen surrounding the occasional projecting chromosome before it can be traced around the entire nucleus. The suggestion is thereby offered that this limiting layer is but a more pronounced expression of the membrane which earlier marked the boundary between the achromatic system of the anaphase chromosome and the cytoplasm.

The question arises as to the disposition of the achromatic material. In the nuclei examined, the karyolymph, at this early stage of its existence as a distinct substance within the newly formed nucleus, shows more affinity for the stains than at later times. A consideration of such a nucleus as that shown in figure 23 shows that the contrast between the chromatic material and the karyolymph is hardly more decided than the contrast

between the two systems of the anaphase chromosomes. The indication of morphologic continuity within the isolated chromosome, as well as the apparent continuity of staining quality, suggests that the karyolymph is a direct product arising from the achromatic material of the chromosomes composing the daughter nucleus. When this material abuts on the cytoplasm a membrane is formed. Where chromosomes are in contact no need for any such membrane arises, and the karyolymph becomes a continuous colloidal system bearing the chromonematic skeletons of the members of the group.

In addition to showing the densely staining karyolymph, figure 23 is valuable in demonstrating the chromonemata during these early telophase changes. It will be noticed that in chromosome *a* the membrane is separated by a narrow zone of karyolymph from the chromonemata and that anastomosing processes run out from the spiral threads to the membrane. Where the contrast of staining of anastomoses and chromonemata is sufficient, the spiral threads can readily be traced and the direction of the twist diagnosed as in this case. But when the contrast is not so decided, as at *b*, the appearance of a reticulum is offered. A sac, such as this, into which the ends of two or more chromosome arms are projecting, offers, by reason of the abundant anastomosing, much difficulty to critical analysis.

With the progress of the reorganization of the daughter nucleus, the chromonemata become more pronounced through the diminution of the affinity of the karyolymph for the color (fig. 24). Delicate attenuations are seen to arise from the slightly fuzzy surface of the chromonemata and pass to connect adjacent chromosomes or even parallel threads of the same one. My observations do not support the contention that these inosculations are adhering portions of chromosomes once pressed closely together. They are outgrowths of the highly active, mobile chromatic threads.

There is an indication in figure 24 as to the origin of the nucleoli. Small, deeply staining granules are shown. They are seen at the ends of the chromosomes and always maintain some connection with them. Larger nucleoli are also obvious, often where the ends of two or more chromosomes are contiguous. Because of their position the smaller granules recall the "polar granules" described for Phrynotettix by Miss Pinney (1908) and Wenrich (1916). The larger nucleoli are apparently compound structures or multiples formed by the union of a series of the smaller bodies. Whether these nucleoli represent unaltered portions of anaphase chromosomes or reservoirs of chromatic material is difficult to determine by these stages. The gradual diminution of the diameter of chromonemata induces the opinion that the latter may be the probable situation. Furthermore, I have not succeeded in distinguishing the detail within these nucleoli which would probably be seen if they contained the two systems found within the anaphase chromosomes. The attachment maintained between nucleolus and chromosome is shown in figure 26, Plate VII.

One of the most diagrammatic representations of the chromonemata and of the polarity of the chromosomes during the telophases is furnished by the nucleus shown in figure 27. It is from a premeiotic cell of a very young microsporangium and is inserted here to illustrate the precise nature of the arrangement of the threads and their relation to the nucleoli.

As the telophases advance, the anastomoses become more pronounced and the outlines of the chromosomes less conspicuous (fig. 28). However, little difficulty is encountered in following the course of the spiral threads, and the hollow-ring appearance of the chromosomes in end views demonstrates the persistence of the cylindric type of structure. Larger nucleoli appear in these nuclei, certainly fusion products of the smaller ones of earlier stages, as is shown by the connecting bridges of figure 29.

A further stage of the development of the anastomoses is seen in figure 30. The chromosome at *a* presents the appearance of a pair of regularly interlaced zigzag threads. Darker spots resembling granules are seen at the points of crossing and bending of the threads. They represent only doubleness or greater amount of thread surface, and not chromomeres. If the latter exist at this time as morphologic units, they are not visible in these preparations.

The chromonemata undergo a gradual diminution of their diameter during the telophase changes. Apparently this diminution is beginning in figure 25, Plate VI, and is quite pronounced in figures 30 and 31, Plate VII.

Up to the time of the formation of the reticulum, in those nuclei in which it is formed, the limits of the constituent chromosomes can be traced with a gradually diminishing degree of preciseness. This is due, not to the loss of morphologic continuity, but to the pronounced inter-chromosomal inosculations. It is also apparent that the nucleus is assuming a more nearly spherical form, a condition which has arisen from the irregularly shaped, many-lobed creation of the early telophase. In the transformation a lengthening of some of the chromosomes must occur, accompanied by much bending and twisting.

Interphases

In the actively dividing meristematic cells mitoses often follow with sufficient rapidity to prevent the intervention of a definite period of rest. This fact is demonstrated by the sparsity of the characteristic resting nuclei as compared with their abundance in the less active or in the older portions of the root. The lobed appearance of many of the prophase nuclei is also suggestive of the shape assumed during the telophases and indicates the failure to proceed to the "resting stage." In a nucleus which has progressed through the telophase changes sufficiently to present a somewhat uniform reticulum, distinction can be made between the constituent chromosomes only occasionally and with difficulty. There is reason to believe that the identity of the chromosomes has not been lost but is only concealed in the reticulum. For example, at *a*, figure 32, the intertwined threads can

be traced. Less favorable examples are offered by other parts of the nucleus where the like affinity of the anastomoses and the chromonemata for the stain prohibit accurate determination. Where the threads appear to cross or where they bend the effect of more deeply staining granules is produced, but these appearances must not be interpreted as chromatic granules supported on a linin network. The linin or achromatic material, as demonstrated by the changes of the telophases, is now to be considered as a part of the karyolymph and continuous with it. Certainly the anastomoses are not to be regarded as a part of the achromatic material, for they, by their origin and persistence, indicate their relation to the chromatin. Larger nucleoli as well as smaller ones are present at this time and usually can be seen to maintain connection by delicate threads with the reticulum and not to lie free in the karyolymph entirely surrounded by a "perinucleolar space."

Prophases

Conceding that only some of the telophase nuclei pass to the "resting stage," it follows that the prophase begins in one case with the chromosome outlines visible and in the other case with them obscured. In those nuclei which have taken on the reticulate type of structure, the first indications of the reappearance of the chromosomes are furnished by a partial withdrawal of the anastomoses (fig. 33). Accordingly, clear spaces come to exist at the places which will mark the boundaries of the chromosomes. Narrow at first, these spaces enlarge as the anastomoses are withdrawn more and more, and the chromosomes become prominent. A consideration of figure 33 reveals that in some portions of the nucleus a definite duality of intertwined parallel threads is visible. It is extremely difficult to understand just why the space between the two threads has become so narrowed since the telophases, unless it is remembered that in the formation of the spherical nucleus lengthening and other movements of the chromosomes have taken place. Approximation of parallel filaments was suggested in figures 26, 27, and 29, but the maximum expression of that affinity is found in these prophase nuclei.

Conspicuousness of the chromosome is enhanced by the additional withdrawal of anastomoses (fig. 34). Here the chromosomes are seen stretched across the nucleus, those nearer the center somewhat more extended than the peripheral ones. The latter seem to be more restricted in their activity as a result of their positions. No differentiation into the chromatic and achromatic materials is visible within each chromosome, so that the chromonemata contrast with the faintly coloring karyolymph. There is no question as to the spiral structure. Ability to trace the coils is sometimes possible; again the *u* or zigzag suggests the type of structure involved. As in the earlier stages, the question of singleness or duality of these filaments is introduced. It will be noted from figure 34 that, although some of the threads do appear as single, there are many proofs of

duality of elements. Numerous interlaced double spirals similar to those observed in the telophases are present (chromosome *a*). Most of these are nearer the periphery than the center of the nucleus and suggest chromosomes that have not undergone a stretching or lengthening. In many of the stretched chromosomes two filaments are observable as delicate parallel threads. That the conspicuous double spiral with the bands widely separated can grade over to the type with the threads almost or quite in contact is demonstrated by figure 35, taken from another nucleus. Chromosomes at this time still maintain connection with the nucleoli (fig. 36). The presence of crypts, crevices, and irregularities in the surface of the latter indicates a gradual transformation of a portion of the contained material.

Nuclei which did not continue to a complete reticulum in the telophases show clearly in the early prophases the limits of the included chromosomes (fig. 37), although some anastomosing is apparent. Continuity of the chromonemata can be traced in some of the chromosomes although it is obscured in others. At a slightly later stage (fig. 38), when the anastomoses are more delicate and not so pronounced, the double spiral threads are discernible. The hollow-ring appearance of the end views is also in evidence (fig. 39). Certain nucleoli, such as the larger one of figure 38, demonstrate their composite nature. The connecting bridges suggest a fragmentation of the larger masses into smaller units (see also fig. 40).

Stretching of the coils is now proceeding rapidly and is accompanied by a certain amount of untwisting. Each of the chromosomes appears as in figure 41, a pair of slender intertwined threads. From these, very delicate pseudopodium-like processes extend out into the karyolymph and across it to the neighboring chromosomes. Such projections are most characteristic surface developments of the chromonemata during the early prophase stages. The threads connecting the chromosomes to the nucleoli are also most pronounced. In the case shown in figure 42 it seems as though the material of the nucleolus were being dissolved, perhaps to be of service in the growth of the chromonemata.

Subsequent development is concerned with the growth of the threads and their uncoiling (fig. 44). The chromonemata at this time are very fuzzy or woolly, numerous processes running out from their surfaces. Some of these traverse the spaces between the parallel threads and appear to connect them. A pair of threads so bridged by anastomoses may at times appear as a single vacuolated band. But it must not be forgotten that the processes extending from the chromonemata are as pronounced or even more pronounced across the inter-chromosomal distances of the karyolymph as across the inter-chromonematic spaces.

As thickening of the threads occurs, it is certain that a shortening and unwinding is also occurring (fig. 45, Pl. VIII). In this nucleus the chromosome stretched across the diameter is already uncoiled, the parallel elements lying side by side, while the more peripherally disposed members still show

the intertwined halves. The anastomoses are decidedly conspicuous, both between the halves of the chromosomes and between the adjacent chromosomes. At this time it becomes apparent that in some of the threads there is an internal distinction between more darkly staining regions and the lighter connecting areas. At a slightly later stage (fig. 46), these chromomere-like regions stand out as more pronounced swellings which bulge beyond the diameter of the thread. Often they appear as paired elements in the parallel chromonemata, although such an arrangement has not been observed as a constant one. No clue as to the possible function of the chromomeres is offered.

In the progression through the prophases the affinity for the stain is gradually increasing, which fact, as in the anaphases, makes it difficult to discern internal structure. There is never a question as to the duality of the elements of the chromosome. The split halves are always pronounced even though they remain twisted around each other until the equatorial-plate stage. The problem of greatest concern is the precise nature of the internal reorganization occurring within each of the threads. Certain of the threads shown in figure 47 give faint indications of containing a pair of spirals (see thread at *a*). The evidence is too slight to be valuable, although I do not doubt that internal differentiation has occurred. However, at the time of the dissolution of the nuclear membrane (fig. 49) one of the half chromosomes shows the detail indicated by figure 50. Certainly there is a distinction here between two substances of different refractive indices and different colloidal characteristics. I found it impossible to trace step by step the internal adjustment which must have been occurring in each of the parallel threads of the prophase chromosome prior to this time. Just how far back the readjustment dates and to what degree organization exists remains a mystery. The first accurate information is offered by figure 48. This projecting end of a late prophase chromosome between the stages represented by figures 47 and 49 shows most pronounced bulges in each thread. Three pairs of these bulges are visible, the upper pairs connected by delicate anastomosing processes. Within each bulge a lighter-colored area is seen, suggesting achromatic material. It is thus apparent, and this observation is verified at subsequent stages, that there are present at this time in each of the chromosome halves both the chromatic and the achromatic systems. It is difficult to determine whether the chromonemata are completely organized. Yet the bulges are highly suggestive of the spiral threads. And again their paired relationship recalls the so-called chromomeres of the telophases and the similar granules of the earlier prophases. In light of this fact, I believe these chromomeres are the first visible evidences of differentiation within the thread, a differentiation which ultimately expresses itself in a distinction between the chromatic and the achromatic substances. The former of these is to appear as the chromonemata of the subsequent generation.

Metaphases

As the nuclear membrane disappears and the chromosomes stretch across the equatorial plate, although their halves may be considerably intertwined they show the undulating outlines suggesting their internal structure. The notched extremities and the hollow-rod appearance in end view are also indicative of the presence of both the chromatic and the achromatic systems. The pronounced median constriction is most strikingly shown in figure 53. Bead-like terminal appendages somewhat suggestive of satellites are seen in figure 51.

Untwisting continues until the halves lie side by side as parallel rods (fig. 54). Structure within these rods is shown in certain parts of this figure but is more clearly portrayed within the single limb seen in figure 55. From a review of many chromosomes at this period it appears probable that at times internal reorganization may still be proceeding, so that a continuous chromonema is not always traceable. It can with certainty be determined, however, that the halves of the chromosomes are not the solid rods usually figured for this stage. They are composed of both chromatic and achromatic materials, with the former visible in the form of a double spiral thread. It follows that, so far as the chromatic material is concerned, the chromosome is a four-parted structure, a pair of parallel halves each containing duality of elements. That the parallel halves will separate during the metaphases has been shown above. The elements within the halves are designed to separate in the metaphases of the succeeding mitosis.

Discussion

Structure of the Chromosomes

During the late prophases, the metaphases, and the anaphases, it is possible to distinguish within a somatic chromosome both chromatic and achromatic materials. Verification of the existence of these dissimilar substances is offered by the metaphase chromosomes of the first meiotic division both in this plant and in *Rhoeo discolor* (figs. 60–65). From such figures as these it is apparent that the oxychromatin is a matrix supporting the basichromatin, and not a core on the surface of which is found the chromatic material. The latter view was held by Bonnevie (1908, 1911) for Ascaris and Allium, de Horne (1911) for Salamandra and Allium, and Vejdovský (1912) for Ascaris.

Sands' aceto-carmine preparations were valuable in demonstrating the achromatic material as the substance in which the chromatin was imbedded. That the latter was peripherally disposed was verified by the living chromosome dissected from the pollen mother cells of this plant by Chambers and Sands (1923), who described an elastic cylinder, possessing a cortex which differs markedly in refractive index from the central core.

Since the oxychromatin is the continuous, bounding substance of the

chromosome, it is not unlikely, as suggested earlier, that a limiting although invisible membrane is present. The chromosome is a complex of systems, colloidal and molecular. At least two of these systems, undoubtedly colloidal, can be distinguished. They have been designated as chromatic and achromatic. The chromosome is in contact with the cytoplasm, another colloidal system. Between the latter two an interfacial membrane is present, undoubtedly not a surface-tension membrane, that is, monomolecular, but of colloidal dimensions. Its existence is most probable, as noted by Seifriz (1921) for other protoplasmic bodies. As this worker has stated, "the chemical and physical forces active in the septum separating two fluid systems are often sufficient to convert the septum into a third system differing physically and chemically from either of the two systems which it separates."

I believe a degree of verification is offered by the series of figures 56–63 showing fixation gradations of a half chromosome during the metaphases of the first maturation division from a solid rod to a pair of parallel spiral threads. Ability of the basichromatin to move freely within the limiting membrane would account for these and similar fixation appearances. In figures 62 and 63 the bounding membrane is removed to some distance from the chromonemata. This condition is caused by the pressure of smearing and is perhaps to be ascribed to the forcing out of the achromatic material, the interfacial membrane being removed from its normal position. There is in these figures the suggestion of a vesicle such as was reported by Janssens (1905) for Batracoseps, Vejdovský (1912) for Ascaris, Lee (1920, 1924) for Salamandra and Paris, and Martens (1922) for Paris. I have failed to find any such vesicle around the somatic chromosomes of Tradescantia. However, the appearance of a vesicle would be simulated if the chromatic material, instead of being appressed to the bounding membrane, were imbedded more deeply in the matrix.

During the telophase changes the achromatin appears to form or to become a part of the karyolymph. Bonnevie and Vejdovský concluded similarly. Consideration of the sacs formed by the ends of chromosome arms (for example, fig. 31, Pl. VII) shows that the karyolymph occupies the same position previously held by the oxychromatin, the membrane of the chromosome being now more pronounced as a nuclear membrane. This membrane of the single nucleus replaces the many limiting layers of the nucleus-like chromosomes.

The Chromonema Theory

In the foregoing pages an attempt was made to trace the chromonemata as persistent structures through the various phases of the mitosis. Schneider (1910) succeeded in this respect, but most authors who have described the chromonema saw the spiral thread of some stages lost or replaced by some other type of structure during the remainder of the nuclear division.

It is fortunate that the observation of the chromonemata by Baranetzky

antedated the ascendancy of the paraffin method, the more so because of the present revolt against conclusions based solely on the coagulation products of fixed material. Baranetzky's analysis of the chromosomes of Tradescantia seems to have lost much of its value with the description of the alternating discs in the same genus by Strasburger (1882) and in other genera by Balbiani (1881), Carnoy (1884), and Korschelt (1884). The association of alternating discs and spiral threads is apparently a close one. Baranetzky saw both but regarded the latter as the correct interpretation. Van Herwerden (1910) compared her preparations of the nuclear thread in the salivary glands of the Chironomus larva, in which she saw spirals, with those of Bolsius (1911) who saw alternating discs. The workers agreed that the thread varied in the different species used and that evidence was offered of the polymorphic structure of the chromatic body. Alverdes (1912) attempted to reconcile the divergent views by finding discs present in very young and very old larvae but double spirals arising from and passing over into the discs. Vejdovský (1912) saw the chromonema originating during the prophases within the then extending thread persisting from the preceding mitosis. But the newly formed spiral broke up into a series of alternating discs prior to the longitudinal splitting of the old chromonema.

The notion that the disrupted, discontinuous chromonema may be the result of certain types of fixation distortion is emphasized by a comparison of the reaction to the aceto-carmine mixture as figured by Sands (1923) and the definite spiral portrayed in this paper. Despite his description of the chromatic material as chromomeres variable in size, shape, and number, Sands figured and mentioned indications of the spiral structure (*cf.* page 62 of the present paper, also Sands' figs. 5 and 10, chromosome *a*). It seems a safe conclusion that the uninterrupted spiral of Baranetzky's report and of this paper, rather than any fragmentation, should be recognized as the normal condition. That any series of artifacts could persistently result in a formation so uninterrupted as the chromonemata appears to be beyond the limits of probability. Wilson (1912) likewise discredited vacuolation, corrosion products, or fixation artifacts as an explanation of the spirals found in the spermatogonial prophases of Orthopteran material.

Sands unfortunately made no attempt to figure the chromosome through the cycle of mitosis. Martens (1922), however, based his interpretation of mitosis in Paris on a discontinuous chromonema. The splitting of the prophase spirem did not occur along but really across the chromonema, in consequence of a bilateral repartitioning of its material. Connections between the half chromosomes were persistent from the repartitioning and not pseudopodium-like outgrowths. Divergent as this interpretation may seem from that herein applied to Tradescantia, and despite the fact that different genera are considered, there seems some basis for comparison. It must be remembered that the double spiral threads have been figured in the telophases of Paris by Lee (1924), although he believed these to be

intertwined halves of the transversely split chromosome. There are certain objections to the application of Martens' interpretation to Tradescantia. (*a*) The chromonemata are not discontinuous, although under certain conditions of artifact they so appear (fig. 5, Pl. VI). (*b*) The splitting of the chromosomes is not a prophase occurrence. (*c*) Processes from the chromonemata not only pass between the threads but also extend out into the karyolymph. (*d*) The prophase chromosomes are tightly interlaced in many cases, even when the halves are most delicate threads (fig. 41, Pl. VII). When they come to lie as extended parallel rods the prophases are well advanced (fig. 44). The threads do not split and then intertwine; they are twisted around each other from the time of their origin.

The literature holds no dearth of description of prophase spirals (Janssens, 1901; Wilson, 1912; Wenrich, 1916; Newton, 1924). It is during the later stages of this period that I have seen the first complete evidence of the endogenous formation of the new chromonemata. They originate within the chromomere-like structures, which accordingly must be centers of greatest physical and chemical readjustments. Whether the occasional paired chromomeres of the telophases (fig. 16, Pl. VI) are perpetuated during interkinesis is a problem still within the realm of the conjectural. It is evident, however, that a remarkable preparation for ensuing mitoses is made far in advance. If the telophase chromomeres are concerned in chromonema-formation, they are related to the spirals becoming visible during the following prophases which will not separate until the metaphases of the succeeding nuclear division. The disruption of the endogenous chromonema of the prophase threads of Ascaris as Vejdovský figured it was a complication associated with the presence of one thread instead of a pair of threads. This complication did much to shatter the force of Vejdovský's argument.

Again, there is no dearth of descriptions of the chromonema during the telophases (Bonnevie, 1908, 1911; Schneider, 1910; Lee, 1920, 1924). Lee's interpretation of intertwined halves of V-shaped chromosomes can not possibly be applied to Tradescantia, since the two threads are visible in each of the limbs of the anaphase chromosome. Lee discounted as "indubitably erroneous" such suggestion of a longitudinal anaphase split, since he saw in Salamandra and Paris a spiral which was a mere rib or surface expression of the chromosome. For the twisting of the halves a polar clumping occurred at the culmination of the anaphase, a condition normally non-existent in Tradescantia.

It is the metaphases and the anaphases which have offered the least confirmation of the chromonema hypothesis. Certainly the spirals are present at this time in Tradescantia, although the difficulty of fixation is greater than during the other phases. The aspect of the quadripartite chromosome of these stages is perhaps but the casual manifestation of the disrupted chromonema. In certain of the preparations of the metaphase

chromosomes of the first meiotic division in Tradescantia, and more especially in Rhoeo, the simulation of a four-parted structure is striking.

Duality of the Chromonemata

There is a striking contrast between the figures of the telophase chromosomes shown in this paper and the irregularly vacuolated structures pictured by Sharp (1920). Some workers who have seen vacuoles have regarded them as median and indicative of a longitudinal split with an ensuing duality of elements. Sharp has suggested that the vacuolation is not median but decidedly irregular. Thus the chromosome of the telophases can be no more called double than triple or quadruple.

On a basis of my preparations, were this irregular telophase vacuolation assumed to occur, I should be forced to account for the disruption and alteration of the two filaments of the anaphase chromosome. That these filaments are actual structures and not the images of the walls of alveoles, as the proponents of the alveolation hypothesis would have us believe, has been proved by certain details of description above.

Too much valuable evidence has been advanced by cytologists working on both plant and animal material demonstrating a longitudinal split during the anaphases or earlier to allow an entire negation of the idea. A most striking additional proof is furnished by the presence of a more or less completely split satellite appendaged from the arm of a late anaphase chromosome of Gasteria (Taylor, 1925). Nor should the fact be overlooked that Bonnevie, one of the advocates of the single-chromonema hypothesis, has figured duality during the anaphases, telophases, and prophases (see her figs. 72, 42, 64). To her this was the unusual and not the normal condition. Yet, is it not more likely that the single thread is a fixation product caused by the lateral approximation of parallel filaments so that the split can no longer be observed?

Disregarding, however, any evidence of anaphase duality, there is for consideration the premise of Sharp that the telophase alveolation is decidedly irregular and that no true duality occurs. As I have traced the changes during the telophase, there are introduced the factors of transformation of the oxychromatin and the translocation of the basichromatin coincident with the thinning of the chromonemata. During the gradual attendant changes it is not impossible to picture the chromosome as an alveolated body if the density of color be the only guide. However, the most diagrammatic intertwined threads have been seen. I am willing to agree that the occasional appearance of a double spiral might readily be explained away on the basis of the aspect of alveoles. But can the appearance of those spirals in a number of chromosomes in a single nucleus be so diagnosed? Another factor which must not be overlooked is the tendency of the chromonemata to send out anastomosing processes. Since these bridge the gaps between the threads, they complicate the analysis of the structural units, cause alveolation appearances, and even obscure the split.

That alveolation is a fixation artifact in this plant is a conclusion emphasized by a consideration of the metaphase chromosomes of the first maturation division shown in figures 56–63, Pl. VIII. Gradation from the evenly staining rod to the double spiral bands is made through a series of magnificently vacuolated structures. Figure 60 as a connecting link clearly portrays how anastomosing between the turns of the coil soon obscures the more precise, correct detail seen in figures 61–63.

The persistence of the dual filaments is rendered questionable by the apparently unsplit threads of some early prophase nuclei (fig. 34, Pl. VII). Were it not possible to find abundant evidence of double structures in these nuclei, the problem would be seriously complicated. However, since only some of the chromosomes, and particularly those stretched across the diameter of the nucleus, have the split obscured, it seems likely that in the stretching and lengthening an approximation of parallel filaments has taken place. This is the stage at which Sharp believed splitting takes place through vacuolation. Lee (1924) abandoned his earlier idea of prophase vacuolation in Paris (1912) in favor of the interpretation of closely intertwined spiral threads. The tendency of parallel threads to approximate to the point of completely hiding the split is well recognized. For example, Sharp's figure 24 of a late prophase in Tradescantia reveals no split, although there is no question of duality at that time in my preparations. The individuality of parallel threads may be concealed to a great extent by the anastomosing processes between them. Proponents of the vacuolation theory regard these processes as residual from the unvacuolated portion of the thread. But it must not be forgotten that the processes connect not only halves but also adjacent chromosomes. Similar projections into the karyolymph have been figured by Bonnevie (1911, fig. 8, Pl. X) and de Horne (1911, fig. 15, Pl. XXXVI).

When the chromonemata are traced through the entire mitosis, it is apparent that they are but parallel halves which differ from the ordinary rods in assuming a coiled form. This phenomenon is perhaps associated with conservation of space, since the coil is a more compact unit than the extended thread. Coincident with the coil is the introduction of chromonema-formation endogenously, a more complicated process than the longitudinal splitting of the rod-shaped structure.

As to any linear arrangement of genes in this plant, the rod-like chromosome can no longer be considered as a unit. Linear arrangement must follow the course of the chromonemata, since they alone maintain the genetic continuity of the chromosome. If the chromonemata are disregarded and the genes are assumed to be located along the greater axis of the chromosome, there would be introduced the possibility of the three-dimensional arrangement suggested earlier by Castle (1919) but more recently discounted by the same author (1921).

Summary

1. The anaphase chromosome is divisible into two morphologically dissimilar substances, one of which, the achromatic, serves as a matrix bearing the other, the chromatic substance. The chromatic substance exists in the form of a pair of unbroken, intertwined spiral threads or chromonemata.

2. At telophase the achromatic substance becomes continuous with the karyolymph, the chromonemata maintaining the genetic continuity of the chromosome.

3. Individuality of the chromosomes and of the chromonemata is apparently not lost during interkinesis.

4. In the early prophases the chromonemata are sometimes seen as distinct intertwined threads, again as a single thread spirally coiled. The latter appearance is probably due to the lateral approximation of parallel filaments sufficient to obscure the split.

5. As the prophases advance, chromomere-like swellings appear in the thread. Each of these can later be demonstrated to be a center of activity concerned in the formation of the new chromonemata.

6. At the equatorial-plate stage the chromosome consists of a pair of parallel threads, each of which shows achromatic and chromatic materials, the latter visible as a pair of spiral threads.

7. Vacuolation, as shown by the metaphase chromosomes of the first meiotic division, is in this plant an appearance caused by artifact, and may be partly due to anastomoses between the chromonemata.

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DESCRIPTION OF PLATES

All observations were made using a Spencer no. 14JP microscope equipped with N.A. 1.40 condenser. All figures were made at the level of the table with the aid of a camera lucida at a magnification of 3,100 diameters, using a Zeiss 2 mm. apochromatic objective and a Zeiss 20x compensating ocular. In reproduction, all except figure 35 are shown at a magnification of approximately 2,050 diameters. Figure 35 is shown at 3,100 diameters.

Figures 1–26, 28–55: Somatic mitoses, *Tradescantia pilosa.*

Figure 27: Premeiotic division, *T. pilosa.*

Figures 56–63: First meiotic division metaphase, *T. pilosa.*

Figures 64, 65: First meiotic metaphase, *Rhoeo discolor.*

Plate VI

Fig. 1. Anaphase: V-shaped chromosome showing narrow median constriction.

Fig. 2. Ditto: Wider constriction.

Fig. 3. Ditto: End view, one arm of the chromosome showing a duality of chromatic elements.

Fig. 4. Ditto: Intertwined chromatic threads or chromonemata.

Fig. 5. Ditto: Discontinuous chromonemata, a fixation distortion.

Fig. 6. Ditto: Chromosomes moving to the poles; note the single pair of terminal satellites.

Fig. 7. Ditto: Terminal indentations suggesting a duality of chromatic elements.

Fig. 8. Ditto: Torn chromosome, verifying the same suggestion.

Fig. 9. Ditto: Chromosome at the stage shown in figure 12, illustrating chromatic and achromatic substances.

Fig. 10. Ditto: Same at a slightly later stage.

Fig. 11. Ditto: Same.

Fig. 12. Late anaphase: Undulating outlines of chromosomes indicative of spiral threads.

Fig. 13. End view of a chromosome arm at the stage shown in figure 12.

Fig. 14. Ditto: Terminal satellite imbedded in an achromatic matrix.

Fig. 15. Ditto: Same.

Fig. 16. Ditto: Portion of a chromosome in which untwisting has occurred. Note the paired chromomere-like swellings.

Fig. 17. Ditto: Grouping of chromosomes at poles.

Fig. 18. Ditto: Apparent fusion of members of the upper group in consequence of density of color and mass of material.

Fig. 19. Detail of chromosome *a*, figure 18.

Fig. 20. Projecting ends of chromosome arms at the same stage.

Fig. 21. Isolated chromosome of the same stage; chromatic and achromatic regions distinguishable.

Fig. 22. Telophase: Satellite on the projecting arm of a chromosome.

Fig. 23. Ditto: Chromonemata persisting, achromatic material becoming continuous with the karyolymph.

Fig. 24. Later stage: Contrast of chromonemata and karyolymph more decided. Anastomoses pronounced.

Fig. 25. Diminution in diameter of chromonemata.

Plate VII

Fig. 26. Telophase: Nucleolus attached to chromosome.

Fig. 27. Ditto: Premeiotic nucleus.

Fig. 28. Ditto: Pronounced anastomosing between chromosomes.

Fig. 29. Ditto: Limits of chromosomes becoming less definite because of anastomosing processes.

Fig. 30. Later stage. At *a*, zigzag aspect of chromonemata.

Fig. 31. Ditto: Zigzag threads in a sac formed by a chromosome arm.

Fig. 32. Interphase: Reticulum simulated by pronounced anastomosing; at *a*, chromonemata visible.

Fig. 33. Prophase: Anastomoses breaking down; duality of threads visible in several places.

Fig. 34. Ditto: Pronounced chromonemata. Chromosome *a* shows double threads; similar appearances in other places.

Fig. 35. Ditto: Showing how a more open spiral can grade over to very close threads.

Fig. 36. Ditto: Nucleolus removed from the nucleus by a knife and carrying with it an attached chromatic thread.

Fig. 37. Ditto: Section of a nucleus which did not form a reticulum, indicating pronounced anastomosing between chromonemata.

Fig. 38. Later stage: Anastomoses withdrawn; compound nucleoli appear.

Fig. 39. End view of chromosomes at the stage shown in figure 38.

Fig. 40. Ditto: Compound nucleolus and attached chromosomes.

Fig. 41. Later stage: Loosening of coils. Pseudopodium-like processes of the surface of chromonemata pronounced.

Fig. 42. Nucleolus from the nucleus of figure 41, showing attached chromosomes.

Fig. 43. End view of chromosomes from the same nucleus.

Fig. 44. Later stage: Uncoiling and thickening of thread.

Plate VIII

Fig. 45. Late prophase: Uncoiling has occurred in some chromosomes but not in others. Anastomoses are pronounced across spaces between halves as well as between chromosomes.

Fig. 46. Ditto: Chromomere-like structures seen.

Fig. 47. Ditto: Shortening and thickening proceeding. At *a*, traces of the formation of the internal spiral.

Fig. 48. Ditto: Chromomere-like structures of earlier stages now concerned in the formation of new spirals. Chromatic and achromatic materials visible.

Fig. 49. Ditto: Chromosomes at the time of the dissolution of the nuclear membrane.

Fig. 50. Half chromosome at a stage comparable to that of figure 49.

Fig. 51. Equatorial-plate stage: Suggestion of satellites.

Fig. 52. Ditto: Halves tightly intertwined.

Fig. 53. Ditto: Pronounced median constriction.

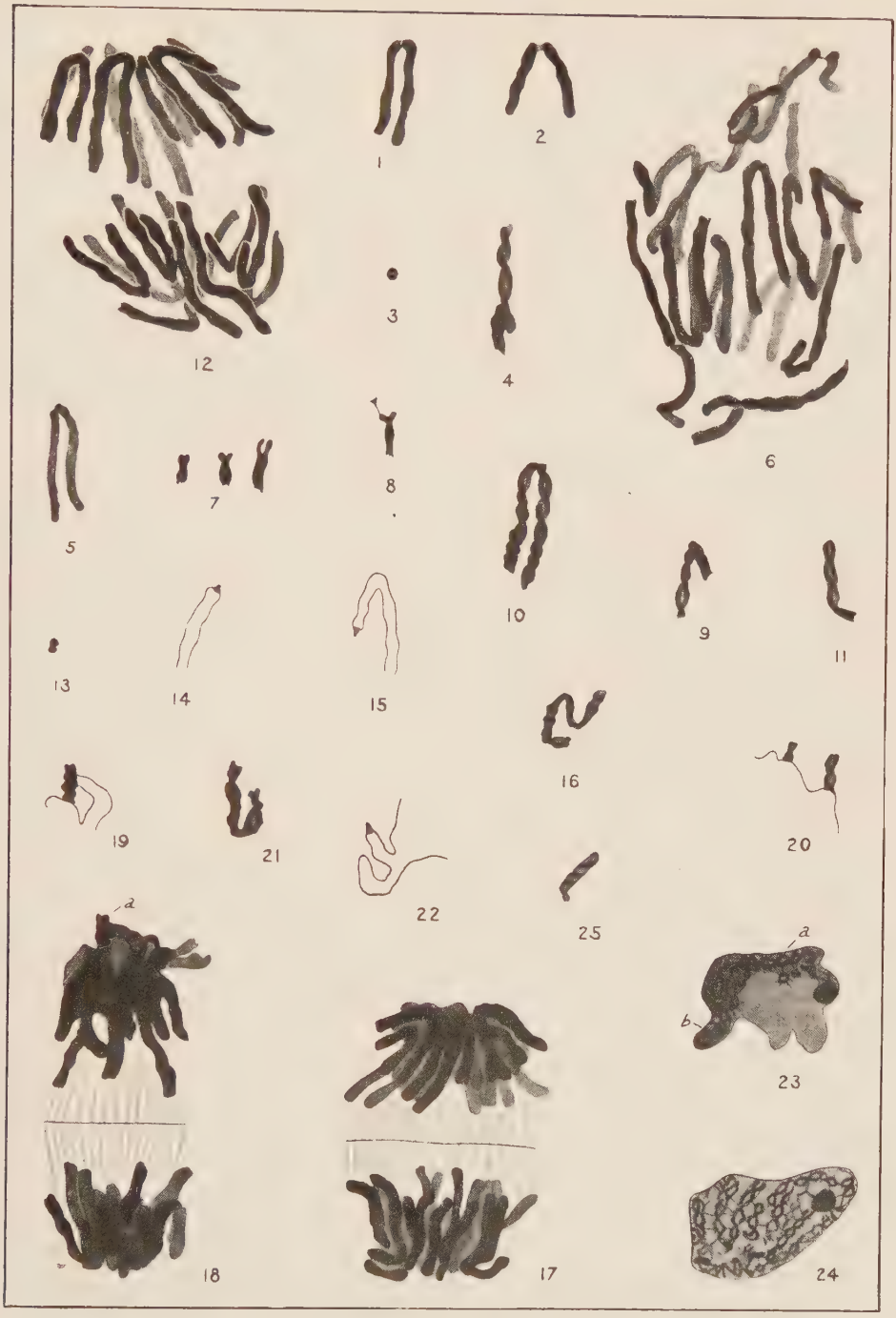
Fig. 54. Ditto: Untwisted chromosomes. In places the halves show internal spiral chromatic threads.

Fig. 55. Arm of chromosome at the stage shown in figure 54, illustrating the chromonemata.

Figs. 56–63. Diads of the first meiotic division. This series shows fixation gradations from the solid rod through vacuolated structures to the single, and eventually the double, spiral threads.

Fig. 64. Diad of a metaphase of the first meiotic division in *Rhoeo discolor*. The achromatic matrix bears chromonemata.

Fig. 65. Tetrad of a metaphase of the first meiotic division in the same plant.



KAUFMANN: CHROMOSOME STRUCTURE

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